A NEW METABOLITE FROM *STREPTOMYCES HYGROSCOPICUS* I. FERMENTATION AND ISOLATION

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A deoxypentulose has been isolated from the fermentations of a new soil isolate of *Streptomyces hygroscopicus* (UC-5601). It was found to inhibit weakly and specifically the growth of one strain of *Mycobacterium avium* (UC-159).

While studying the fermentations of a new soil isolate of *Streptomyces hygroscopicus* (UC-5601), a new substance inhibiting specifically one strain of *Mycobacterium avium* (UC-159) was found to be produced in addition to hygromycin A and hygromycin B. Since the family of hygromycin antibiotics is rather large, comprising hygromycins A, B, C and compounds D, E, F,[1] we considered it of interest to isolate this bioactive material and to establish its relation to the described hygromycin antibiotics. The compound was isolated from the fermentations in crystalline form and its structure was established as 1-deoxy-D-*threo*-pentulose by HOEKSEMA and BACZYNSKYJ.[2]

The fermentation conditions and the isolation of this sugar from the fermentation broth are the subject of this paper.

I. Microorganism

The antibiotic producing microorganism was a new isolate identified as *Streptomyces hygroscopicus* (UC-5601) by Ms. ALMA DIETZ of The Upjohn Company.

II. Analytical Methods

The fermentation media and various fractions obtained during the isolation procedure were analyzed by thin-layer chromatography on silica plates (Uniplate, Analtech, Inc.) developed in acetone-ethylacetate (1:1) and subsequent bioautography using *Mycobacterium avium* (UC-159). Three bioactive spots were detected. Two were identified as hygromycin A (Rf=0.2) and hygromycin B (Rf=0.0). The third substance (Rf=0.42) was considered to be a new activity. Good separation was also obtained on thin-layers of cellulose (Eastman Kodak) with butanol-1-water (84:16) +2% piperidine as the solvent system.

The total antibiotic production was determined by paper-disc agar diffusion assay employing *M. avium* as test organism.

III. Fermentation

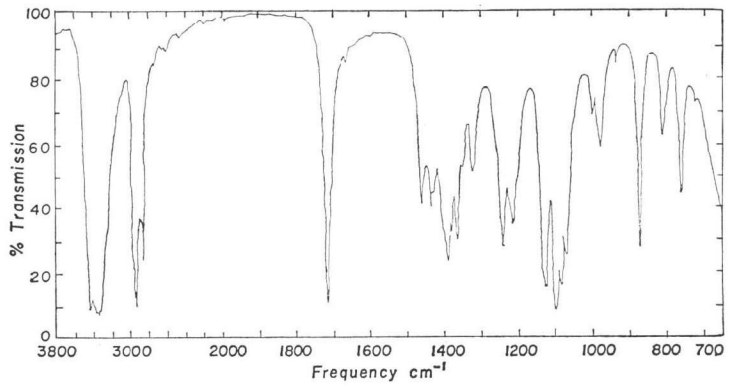
Seed cultures of *S. hygroscopicus* were prepared in a medium composed of (in g/liter): Bacto-peptone (Difco), 10.0; Bacto-yeast extract (Difco), 2.5; glucose monohydrate, 10.0. The cultures were incubated at 28°C for 96 hours on a rotary shaker. The fermentation medium had the following composition (in g/liter): glucose monohydrate, 20.0; Buffalo starch (CPC International) 20.0; brewers yeast (Philadelphia Dried Yeast Co.), 3.5; Kay Soy (Archer Daniels

Midland Co.), 2.0; ammonium sulfate, 0.75; calcium carbonate, 4.0; adjusted to pH 7.2. Fermentations inoculated with 5% seed culture were incubated at 32°C on a rotary shaker and harvested after 96~120 hours.

IV. Isolation Procedure

The isolation procedure used in this work was essentially an adaptation of the method described by ELBEIN *et al.*[8] The filtered fermentation broth was reduced to one half of the original volume *in vacuo* and solid ammonium sulfate was added to make a saturated solution which was extracted twice with 0.7 volumes of butanol-1. Thin-layer chromatography and subsequent bioautography of an aliquot of the combined butanol-1 extracts revealed the presence of hygromycin A (Rf=0.20) and the new activity (Rf=0.46). Under these conditions, hygromycin B was not extracted and remained in the water phase. The butanol-1 extract was reduced to one tenth of the original volume under reduced pressure and the precipitated ammonium sulfate was filtered off. Six volumes of petrol ether were added to the clear butanol-1 solution and after standing overnight at 5°C, the precipitate containing the hygromycin A was removed by filtration. The clear filtrate was reduced to brown oil by removal of the solvent *in vacuo*. Thin-layer chromatography and bioautography of this material showed the presence of only one biologically active substance, namely the new activity (Rf=0.46). The bulk of this material was dissolved in water (5 g in 100 ml), the insoluble impurities were filtered off and the clear, brown, solution was decolorized by passing it through a column of activated carbon. The colorless solution was evaporated *in vacuo* to yield a yellow oil which was triturated with excess (20 fold) of acetone. After separation of the insoluble material, the acetone solution was evaporated and the oil thus obtained, after standing at 5°C for a day, solidified as light brown crystalline material. Finally, the crystalline material was treated with boiling ethyl acetate (100 ml of solvent per 10 g of solids) and the pale yellow solution was decanted from the brown oil. Upon standing for 2 days at 5°C, the white crystalline material formed was isolated by filtration, washed with ether and dried *in vacuo*. After reduction of the volume of the mother liquor to one half, a second crop of crystalline material was obtained. Starting with 5,000-liter fermentation and following the above procedure, 381 g (first crop) and 61 g (second crop) of crystalline substance were isolated. An analytically pure sample was obtained by recrystallizing the first crop crystals twice from boiling ethyl acetate; *Anal.* Calculated for $C_5H_{10}O_4$; C 44.77,

Fig. 1. Infrared spectrum of 1-deoxy-D-*threo*-pentulose (mineral oil mull).



H 7.51. Found: C 44.98, H 7.85, mp 61~63°C, $[\alpha]_D$+46° (*c* 1, H_2O). The infrared spectrum shown in Fig. 1 is indicative of a compound with a high percentage of hydroxyl function (3300~3450 cm^{-1}), and a very pronounced carbonyl absorption as 1710 cm^{-1}.

V. Biological Properties

Crystalline 1-deoxy-D-*threo*-pentulose was found to inhibit *in vitro* the growth of our test strain of *M. avium* (UC-159) at 1 mg/ml. However, it was inactive at 20 mg/ml against the following species of *Mycobacterium*: *phlei* (UC-3092), *fortuitum* (UC-3405), *rhodochrous* (UC-3083), *smegmatis* (UC-3892), *kansasii* (UC-3857), *avium* (UC-3518) and BCG. This compound was also inactive against gram-positive and gram-negative bacteria, fungi and mouse leukemias L-1210 and P-388 *in vitro*. No toxicity was demonstrated in mice following subcutaneous and oral administration.

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